Testing the Effects of Chemicals on Earthworms: The Advantages and Limitations of Field Tests

C.A. EDWARDS

The Ohio State Unversity, Columbus, Ohio, USA

Introduction

The soil is one of our most valuable natural resources, so the maintenance of soil fertility is of major importance. The main factor promoting soil fertility is the breakdown of dead organic matter and the release of the nutrients that it contains. The main agents in these processes are the multitudes of soil organisms that are characteristic of healthy soils. Hence, it is extremely important that populations of soil-inhabiting micro-organisms and invertebrates be maintained. Earthworms are probably the most important soil-inhabiting invertebrates in promoting soil fertility (Edwards and Heath, 1964).

Some pesticides and industrial chemicals can have drastic effects on soil organisms; hence it is important to know the relative toxicity of chemicals that may reach soils, so that the exposure of soil organisms to the more toxic chemicals can be avoided or minimised. The various organisms that inhabit soils differ greatly in their susceptibility to chemicals (Edwards, 1973; Edwards and Thompson, 1973; Thompson and Edwards, 1974) so it is very difficult to assess the overall effects of different chemicals on dynamic soil processes that are mediated by soil organisms. Earthworms are extremely important in the breakdown of organic matter into soil, the release of nutrients, the aeration and drainage of soil and the formation of soil aggregates.

Earthworms have a number of characteristics which identify them as one of the most suitable soil animals to be used as a key bioindicator organism to assess soil pollution by chemicals. In addition to their key role in soil fertility, they are common in the great majority of soils, they are large in size and easy to handle, they can be readily collected and identified and are known to be affected by and take up into their tissues, a number of organic and inorganic chemicals.

The effects of chemicals on earthworms and methods of investigating these effects have been reviewed by Satchell (1955), Davey (1963), Edwards and Thompson (1973), Thompson and Edwards 1974) and Lofs-Holmin and Boström (1988), Edwards and Neuhauser (1988).

Most of the earlier reports in the scientific literature refer to field testing of the effects of chemicals, particularly pesticides, on earthworm populations. These are too numerous to list (Edwards and Thompson, 1973) but range from the effects of lead arsenate (Polivka, 1951; Escritt, 1955) copper sulphate and organochlorines (Davey, 1963; Edwards *et al*, 1967) carbamate compounds (Thompson, 1971; organophosphorus compounds (Edwards *et al*, 1968; Thompson, 1970; Way and Scopes, 1968) and herbicides (Edwards, 1970). When the results of these field experiments are reviewed it is extremely difficult to assess reliably the relative toxicity of different pesticides, due to considerable variability between test sites, soils, formulations, doses and methods of application used. The field tests reported in the literature are adequate to identify chemicals which are extremely toxic to earthworms but do not identify accurately, moderately or slightly toxic compounds (Lofs-Holmin and Boström, 1988; Edwards and Bohlen, in press). There are at least 250 reports in the literature on the testing of effects of chemicals on earthworms, either alone or in combination with other organisms in field tests. These tests have been extremely variable in design and execution and the results are extremely difficult to compare and assess (Lofs-Holmin and Boström, 1988; Edwards and Bohlen, in press) in terms of potential environmental hazard.

There are many methods of testing the effects of chemicals on earthworms in the laboratory and one of these, the artificial soil test (Edwards, 1983, 1984; Goats and Edwards, 1988) has been adopted by the EEC and OECD as a standard test. However, there is an urgent need for a standardised field test so that laboratory and field test results can be correlated, with the ultimate aim of using the laboratory test for hazard prediction without the need for expensive field tests.

The aim of this paper is to review the advantages and limitations of field tests for assessing the toxicity of chemicals to earthworms and to make suggestions on a standardised design for such field experiments.

Advantages of Field Tests for Assessing Toxicity of Chemicals to Earthworms

Most of the earliest reports, in the literature of the effects of chemicals on earthworms, involved pesticides and were aimed primarily at assessing the effects of these chemicals on earthworm populations, with the overall objective of avoiding the use of pesticides that were very toxic to earthworms.

Field tests that assess the toxicity of chemicals to earthworms have some obvious advantages. The exposure of earthworms to the chemicals is identical to that to which they are exposed after the application of agricultural chemicals to soils or when industrial chemicals fall on the surface of the soils. Moreover, the exposure of the earthworm to chemicals is under natural climatic and environmental conditions. In field tests a range of development stages of earthworms are exposed simultaneously to chemicals and the toxic effects of the chemical are superimposed on natural mortality and predation. By comparison, most laboratory tests involve a single life stage of earthworm, natural mortality is minimised and the test worms are in optimal health. In the field a range of species of earthworms are exposed to chemicals simultaneously, compared to the single species used in laboratory tests that may not be totally representative of the general population. In field tests, if a sequential series of samples is taken in time, the longevity of the toxic effects of the chemical under field conditions can be assessed, compared with a relatively short period of exposure to chemicals that is possible under the standardised conditions in the laboratory.

Most laboratory tests are designed to assess only the acute or chronic direct toxicity of a chemical. By comparison, field tests can also evaluate the indirect effects of chemicals. These include: effects of the chemical on the earthworm's food supply, eg. herbicides affect the amount of decaying plant material in soil, and fungicides may influence populations of fungi that are a major component of the diet of many species of earthworm (Edwards and Fletcher, 1988). Similarly, chemicals can have indirect effects on the earthworm habitat, eg. herbicides affect the degree of soil cover which keeps the surface soil much moister.

Earthworms can take up many chemicals from soil into their tissues, and in field experiments the exposure is under a range of different environmental conditions. By comparison, the exposure in the laboratory can only be under selected constant conditions. For example, in the field, aldicarb is taken up into earthworm tissue only in the form of the parent compound in waterlogged soils and not as the degradation product. Such a type of differential uptake would not be determined in laboratory experiments under controlled environmental conditions.

Clearly, field experiments have many advantages in the assessment of potential hazards from chemicals but there is an urgent need for a standardised design of field experiments and defined criteria for assessing results if they are to be effective.

Limitations of Field Tests for Assessing Toxicity of Chemicals to Earthworms

FACTORS THAT INFLUENCE THE EXPOSURE OF EARTHWORMS TO CHEMICALS

The amount of exposure of earthworms to chemicals in soils depends upon a wide range of variable *factors associated with the chemical*, the soil type, the environmental conditions, and the behaviour of the earthworms. Chemical characteristics ~~that~~ affect their impact upon earthworms. Chemical characteristics that affect their impact upon earthworms include: volatility, water solubility, lipid/water partition coefficient, adsorptive capability and persistence in soils.

Various *characteristics of soils* can influence the toxicity to earthworms of chemicals that reach it. When soil has a low moisture content, the toxicity of some chemicals can be decreased through adsorption on to soil fractions, whereas compounds that dissolve readily in water can be leached from a soil that is water-logged. Chemicals can bind reversibly or irreversibly with the particles of clay and organic matter in the soil, and the proportions of these two components in soil will also influence the cation exchange capacity of the soil, which will affect the amount of chemical that is available to the earthworms. Soil temperature affects the vapour pressure of a chemical and also its solubility in soil water, thereby influencing the rate at which compounds are taken up by earthworms. The rates of loss of chemicals from the soil as a vapour or through the action of soil micro-organisms also depends upon soil temperature. Micro-organisms are instrumental in detoxification and degradation of many chemicals, yet the range of detoxification and degradation of many chemicals, yet the range of micro-organisms present in the soil at an experimental site, and their subsequent activity, is seldom known because it is difficult to determine and standardise.

The *behaviour of earthworms* can affect their exposure to chemicals in the field. The horizontal distribution of earthworms in the soil is limited by certain aspects of the physicochemical environment, such as the temperature, moisture content and pH of the soil, the availability of food and the capacity of the earthworms to reproduce and disperse. Vertical distributions of earthworms are also affected by changes in the soil conditions, such as temperature and moisture (Edwards and Lofty, 1977) and the ability of the earthworms to respond to these changes. Juvenile earthworms are often unable to burrow deeply in the soil and are therefore affected more severely than the adults by toxic chemicals. By contrast, adult earthworms of deep burrowing species can move rapidly into the safety of the deep soil layers using permanent, mucous-lined burrows. Earthworms can move into diapause under adverse temperatures or moisture conditions.

Different earthworm species can be exposed to quite different amounts of chemicals in different ways. For instance, *L. terrestris* is often exposed to a high concentration of pesticides, because this species of earthworm moves on and feeds on the soil surface. *A. caliginosa* lives in the superficial layers of the soil and the adults move on the soil surface in wet weather, becoming particularly vulnerable to surface pesticide residues. *A. longa* seems to be less susceptible to pesticides than many other species of earthworm, because it can burrow deep into the soil (Wheatley and Hardman, 1968) and enters an obligatory diapause during the summer (Gerard, 1967; Edwards and Lofty, 1977).

Both *L. terrestris* and *A. longa* construct permanent burrows (Edwards, 1983) and this probably decreases the exposure of these earthworms to pesticides that are incorporated into the soil. However, under some conditions such burrows can channel chemicals that are drenched onto the soil, allowing them to percolate rapidly and make rapid contact with the earthworms.

Earthworm activity affects exposure. For instance, temperate earthworm species have a peak period of activity from April to May and from August until early December. *L. terrestris* appears to become inactive in summer (Gerard, 1967), and *A. longa* enters an obligatory diapause from May until

October, so *L. terrestris* and *A. longa* have similar seasonal behaviour patterns. *A. caliginosa, A. chlorotica* and *A. rosea* can enter a facultative diapause during unfavourable conditions (Evans, 1947) and such resting behaviour can affect results of field tests. The activity of earthworms in soil is limited mainly by temperature and moisture, although drought induces a more profound quiescence than winter cold (Gerard, 1967). The optimal temperature for earthworm activity can differ between species. *A. caliginosa* and *E. fetida* are most active in soils between 10–23°C and 16–23°C respectively, whilst *L. terrestris* is more active in soil at 10°C (Edwards and Lofty, 1977). High temperatures tend to limit the activity of earthworms more than low temperatures. The removal of an insulating layer of vegetation from the field cultivations can increase the range within which the temperature of the soil fluctuates, and thus affect populations of earthworms adversely (Edwards, 1984).

VARIABILITY IN FIELD EXPERIMENTS ON THE TOXICITY OF CHEMICALS TO EARTHWORMS

The field experiments which have assessed the effects of chemicals on earthworms in the literature are extremely diverse and poorly-designed (Lofs-Holmin and Boström, 1988). As many as 7% of field experiments had *no control or check* plots other than by a loose comparison with earthworm populations in fields close to the one treated with chemicals. Many experiments in the literature have inadequate replications which would enable statistical analyses of the data to be made.

More than a third of the field experiments reported in the literature either had *no replicates* (12%) or gave inadequate information on the degree of replication (24%). About half had 2–4 replicates and the rest had 5-10 replicates. Nearly a quarter of the field experiments did not define *the dose*, other than to state that it was 'recommended', 'commonly-used' or 'normal'.

Little information on the *soil type* or its characteristics was given in most field experiments. Less than half of them reported the soil pH, texture or organic matter content. Very little information on *climatic variation* during the exposure period was given and the periods of exposure to the chemical were very variable, most lasting 1–6 months but some continued for up to 5 years. A few of the experiments reported lasted less than one month.

The effects of chemicals on earthworms was assessed using *extremely variable critera*. These include: assessment of earthworm populations (as numbers or biomas) by hand-sorting, pitfall traps, formalin extraction, counting dead earthworms on the soil surface, counting earthworm casts, measuring the concentration of the chemical in earthworms, assessing the consumption of organic matter from the surface, or counting earthworm burrows. Some of these indices are affected by seasonal activity, the behaviour of earthworms and a variety of environmental factors.

Clearly, if field tests for assessing the toxicity of chemicals to earthworms are to be useful and comparable, there must be much more standardisation in their design and procedures.

The Design of Standardised Field Experiments

There is bound to be some variability in the results of field experiments, due to differences in soil type, climatic factors and species and numbers of earthworms present. However, field experiments can provide valuable data that is almost impossible to obtain from laboratory experiments, if a carefully standardised design of field experiment is used.

The following suggestions for the design of field experiments to assess the toxicity of chemicals to earthworms in the field can be made.

SITE VARIABLES

It is important to choose a site carefully. The important factors influencing this choice are:

- A large population of earthworms with a representative range of *at least six species of common earthworms*. Preferably at least 100 earthworms per m^2 should be present prior to application of the chemical, although a lower population does not preclude the sue of a site; it means that more samples will be needed. Such populations can be found in many arable soils that have not been exposed to pesticide treatments, in temperate regions. *Lumbricas terrestris* is probably the most important species, because it incorporates organic matter into soil, it has permanent burrows through which chemicals can percolate, and it becomes exposed to surface residues of chemicals more often than other species, so the test site should have this species if possible. *A. caliginosa* is probably the most important shallow-working species and should preferably be present.
- The *soil-type* should be preferably loam-based, avoiding extreme clay soils which bind up chemicals and sandy soils which do not favour earthworms. The soil type should be as homogeneous as possible across the experimental site. The classification and description of the soil type and its organic matter content should be included in all reports.
- As far as possible there should be *no history of chemical use on the site for at least 5 years* prior to the experiment. An analysis of the soil for chemical residues prior to the experiment is recommended.

TREATMENT VARIABLES

The way in which the chemical reaches the earthworm influences its effect greatly:

- The *chemical* should be clearly defined in terms of structure and mix of isomers. If it is a pesticide its common name should be known. Its physicochemical properties such as solubility, water/lipid partition coefficient and volatility, which can be used in prediction of toxicity should be known. Its formulation which also influences its toxicity should be stated.
- The *dose* used should be relevant to the exposure likely to occur under natural conditions. For agricultural chemicals, it should be the highest

recommended dose (kg/ha active ingredients). For industrial chemicals it should parallel amounts reported in environmental surveys. If higher doses are tested they should be in the series 2x, 4x, 8, 16x, the recommended dose, to enable dose/effect curves to be plotted.

– The *chemical should always be applied to replicated plots*. As a general guide a minimum of four replicates should be used. The greater the number of replicates the greater the precision of the experiment.

– The *size of plot* is important. Earthworms, other than *L. terrestris* do not migrate far laterally, so relatively small plots with no physical barriers between them are adequate. Plots should be at least 5 metres square and up to 10 metres square. With plots larger than this, the potential variability of the soil type over a large area can increase the variability of the results. There is considerable evidence that earthworms do not migrate significantly between plots of these sizes (Edwards, 1973 a&b).

– *the cropping of the experimental site should be defined*. If a chemical is tested that would be expected to reach the ground in fall-out or precipitation it would be appropriate to apply the test chemical to grassland that contains a suitable earthworm population. However, if a chemical is to be tested that would normally be applied to arable land, it should be applied to bare soil to avoid binding the pesticide to the surface thatch commonly found in grassland. Preferably, it should be an arable site with a suitable earthworm population. After the chemical has been applied, the plots can then be seeded to grass or other crops. Row crops should be avoided since they complicate the assessment of earthworm populations. The testing of herbicides in the field is more difficult because the indirect effects of the herbicide on earthworms through changed weed populations may be greater than the direct effects of the chemical so, to assess direct toxicity, plots must be kept fallow (Edwards and Stafford, 1978). When an arable site with an adequate earthworm population is not available, an alternative is to cultivate a grassland site and preapre a seedbed before applying the test chemical.

– The *timing of the experiment* is very important. In temperate countries earthworms are most active in spring and autumn. It is recommended that whenever possible, experiments should begin in spring and should all be continued for a minimum of six months to include the autumn months, but longer (up to two years or more) for persistent chemicals.

– It is recommended that whenever possible the effects of a test chemical should be compared with those of a *toxic standard*. Possible standard chemicals include benomyl or carbaryl (Edwards 1983, 1984).

– If the issue of entry into food chains is to be addressed, residues of the chemical in earthworms and soil should be measured to assess whether *bioconcentration* occurs (Edwards, 1973 a&b). Methods of assessing residues in worms are described elsewhere (Edwards, 1979).

ASSESSMENT CRITERIA

The most important criteria in assessing the overall effects of a chemical on earthworms is the *overall effect on populations* (numbers and biomass) of earthworms. The assessment of populations of soil-inhabiting invertebrates such as earthworms is extremely difficult (Edwards, 1991).

The most thorough *method of assessing populations* of earthworms, to include all stages of the population (cocoons, immatures, resting and active adults), is by hand-sorting soil sample from a quadrat (0·25–1·0 m^2) to a depth of 30–60 cm in the field or laboratory. However, this is extremely laborious and time-consuming.

An alternative method of assessing populations in the field is by using quadrates (0·25–1·0 m^2) and pouring litres of dilute formalin (50 cc of 40% formaldehyde in 9 litres of water) on to the soil gradually. Earthworms will come to the surface within 15–20 minutes, can be picked up with forceps and stored in 5% formalin until they can be identified. This method is relatively quick but has the drawback that it works best when earthworms are at peak seasonal activity and is most effective for those earthworms with permanent burrows such as *L. terrestris* and *Allolobophora longa*. This method does not sample cocoons or diapausing worms.

The method recommended for maximum efficiency is to hand-sort earthworms in the top 5–10 cm of soil in the field, clear this soil from the quadrat and then pour dilute formalin on to it. This method recovers both these earthworm species that live most of their lives close to the soil surface (endogeic), as well as those that penetrate as much as 2 metres deep into the soil through burrows (anecic) and therefore would not be recovered by hand-sorting.

It has been suggested that an electrical method of sampling by passing a current from a 220 volt generator through soil is a suitable sampling method (Satchell, 1955; Edwards and Lofty, 1977; Thielemann, 1986). However, this method does not sample a sharply delimitated area of soil and is greatly influenced by soil pH and moisture content.

– The *timing of earthworm sampling* in field experiments should be related to the persistence of test chemicals in soil, as far as it is known. For chemicals that persist less than six months in soil, monthly sampling is adequate. For chemicals that persist longer it is recommended that samples should be taken 1,2,4,8,12 and 24 months after treatment with sampling every six months after that for very persistent chemicals.

– The *number of earthworm population samples* needed per replicate can differ with variations in soil type, poulations of earthworms and size of plot. However, it is recommended that a minimum of four samples per plot be taken on each sampling date if a statistical change in population of the order of 25% is to be identified.

Conclusions

The value of field experiments in assessing the effects of chemicals upon earthworms can be increased enormously by using carefully designed experiments. We need much more data from well-designed and standardised field experiments that can be compared with data for the same chemical from recommended dose (kg/ha active ingredients). For industrial chemicals it should parallel amounts reported in environmental surveys. If higher doses are tested they should be in the series 2x, 4x, 8,x 16x, the recommended dose, to enable dose/effect curves to be plotted.

– The *chemical should always be applied to replicated plots*. As a general guide a minimum of four replicates should be used. The greater the number of replicates the greater the precision of the experiment.

– The *size of plot* is important. Earthworms, other than *L. terrestris* do not migrate far laterally, so relatively small plots with no physical barriers between them are adequate. Plots should be at least 5 metres square and up to 10 metres square. With plots larger than this, the potential variability of the soil type over a large area can increase the variability of the results. There is considerable evidence that earthworms do not migrate significantly between plots of these sizes (Edwards, 1973 a&b).

– *the cropping of the experimental site should be defined*. If a chemical is tested that would be expected to reach the ground in fall-out or precipitation it would be appropriate to apply the test chemical to grassland that contains a suitable earthworm population. However, if a chemical is to be tested that would normally be applied to arable land, it should be applied to bare soil to avoid binding the pesticide to the surface thatch commonly found in grassland. Preferably, it should be an arable site with a suitable earthworm population. After the chemical has been applied, the plots can then be seeded to grass or other crops. Row crops should be avoided since they complicate the assessment of earthworm populations. The testing of herbicides in the field is more difficult because the indirect effects of the herbicide on earthworms through changed weed populations may be greater than the direct effects of the chemical so, to assess direct toxicity, plots must be kept fallow (Edwards and Stafford, 1978). When an arable site with an adequate earthworm population is not available, an alternative is to cultivate a grassland site and preapre a seedbed before applying the test chemical.

– The *timing of the experiment* is very important. In temperate countries earthworms are most active in spring and autumn. It is recommended that whenever possible, experiments should begin in spring and should all be continued for a minimum of six months to include the autumn months, but longer (up to two years or more) for persistent chemicals.

– It is recommended that whenever possible the effects of a test chemical should be compared with those of a *toxic standard*. Possible standard chemicals include benomyl or carbaryl (Edwards 1983, 1984).

– If the issue of entry into food chains is to be addressed, residues of the chemical in earthworms and soil should be measured to assess whether *bioconcentration* occurs (Edwards, 1973 a&b). Methods of assessing residues in worms are described elsewhere (Edwards, 1979).

ASSESSMENT CRITERIA

The most important criteria in assessing the overall effects of a chemical on earthworms is the *overall effect on populations* (numbers and biomass) of earthworms. The assessment of populations of soil-inhabiting invertebrates such as earthworms is extremely difficult (Edwards, 1991).

The most thorough *method of assessing populations* of earthworms, to include all stages of the population (cocoons, immatures, resting and active adults), is by hand-sorting soil sample from a quadrat (0·25–1·0 m^2) to a depth of 30–60 cm in the field or laboratory. However, this is extremely laborious and time-consuming.

An alternative method of assessing populations in the field is by using quadrates (0·25–1·0 m^2) and pouring litres of dilute formalin (50 cc of 40% formaldehyde in 9 litres of water) on to the soil gradually. Earthworms will come to the surface within 15–20 minutes, can be picked up with forceps and stored in 5% formalin until they can be identified. This method is relatively quick but has the drawback that it works best when earthworms are at peak seasonal activity and is most effective for those earthworms with permanent burrows such as *L. terrestris* and *Allolobophora longa*. This method does not sample cocoons or diapausing worms.

The method recommended for maximum efficiency is to hand-sort earthworms in the top 5–10 cm of soil in the field, clear this soil from the quadrat and then pour dilute formalin on to it. This method recovers both these earthworm species that live most of their lives close to the soil surface (endogeic), as well as those that penetrate as much as 2 metres deep into the soil through burrows (anecic) and therefore would not be recovered by hand-sorting.

It has been suggested that an electrical method of sampling by passing a current from a 220 volt generator through soil is a suitable sampling method (Satchell, 1955; Edwards and Lofty, 1977; Thielemann, 1986). However, this method does not sample a sharply delimitated area of soil and is greatly influenced by soil pH and moisture content.

– The *timing of earthworm sampling* in field experiments should be related to the persistence of test chemicals in soil, as far as it is known. For chemicals that persist less than six months in soil, monthly sampling is adequate. For chemicals that persist longer it is recommended that samples should be taken 1,2,4,8,12 and 24 months after treatment with sampling every six months after that for very persistent chemicals.

– The *number of earthworm population samples* needed per replicate can differ with variations in soil type, poulations of earthworms and size of plot. However, it is recommended that a minimum of four samples per plot be taken on each sampling date if a statistical change in population of the order of 25% is to be identified.

Conclusions

The value of field experiments in assessing the effects of chemicals upon earthworms can be increased enormously by using carefully designed experiments. We need much more data from well-designed and standardised field experiments that can be compared with data for the same chemical from standardised laboratory experiments in order to test the validity of the laboratory test. However, the results should be used whenever possible combined with full knowledge of the physico-chemical characteristics of the compound under test and the results of at least a simple laboratory test (Figure 1) (Edwards, 1990). By combining the results from the field and laboratory it is much easier to reach a balanced judgement on the potential toxicity and environmental hazard of a compound. As a suitable data bank is accumulated involving not only physico-chemical data, but also the results of laboratory and field experiments for chemicals with particular structures and properties, accurate prediction of particular hazards to earthworms should become much more feasible.

References

DAVEY, S.P. (1963). Effect of Chemicals on Earthworms: A Review of the Literature. USDI Special Scientific Report Wildlife No 74, Washington USA, pp 20.

EDWARDS, C.A. (1973a). Environmental Pollution by Pesticides. Plenum Press, London and New York, pp 542.

EDWARDS, C.A. (1973b). Persistent Pesticides in the Environment. 2nd Edition. CRC Press, Cleveland, OH. pp 170.

EDWARDS, C.A. (1979). Tests to Assess the Effects of Pesticides on Beneficial Soil Organisms. Proc. Umweltbundsamt Seminar on Ecological Tests Relevant to Environmental Chemicals: Evaluation and Research Needs. December 1977, 240–253.

EDWARDS, C.A. (1983). Development of a standardised laboratory method for assessing the toxicity of chemical substances to earthworms. Report of Commission of European Communities. EUR.

EDWARDS, C.A. (1984). Report of the second stage in development of a standardised laboratory method for assessing the toxicity of chemical substances to earthworms. Report for Commission of European Communities. EUR 9360 EN. pp 99; 8714 EN. pp 141.

EDWARDS, C.A. (1991). Methods for assessing populations of soil-inhabiting invertebrates. Agriculture, Ecosystems and Environment **34**, 145–176.

EDWARDS,C.A. AND BATER, J.E. (1990). An evaluation of laboratory and field studies for the assessment of the environmental effects and pesticides. British Crop Protection Conference, Pests and Diseases, 963–968.

EDWARDS, C.A. AND BOHLEN, P. (1991). The Assessment of the Effects of Toxic Chemicals Upon Earthworms. In: Reviews of Environmental Contamination and Toxicology. Ed G. Ware. (in press).

EDWARDS, C.A., DENNIS, E.B. AND EMPSON, D.W. (1967). Pesticides and the soil fauna effects of aldrin and DDT in an arable field. Annuals of Applied Biology, **60**, 11–22.

EDWARDS, C.A. AND FLETCHER, K.E. (1988). Interactions Between Earthworms and Microorganisms. Agriculture, Ecosystems and Environment, 235–249.

EDWARDS, C.A. AND LOFTY, J.R. (1977). Biology of Earthworms. 2nd Edition, Chapman and Hall, London, pp 333.

EDWARDS, C.A. AND NEUHAUSER, E.F. (ED.) (1988). Earthworms in Waste and Environmental Management. SPB Academic Publishing, The Hague, The Netherlands, pp 391.

EDWARDS, C.A. AND STAFFORD, C.J. (1978). Interactions Between Herbicides and the Soil Fauna. Annuals of Applied Biology, **89**, (2), 91, 125–146.

EDWARDS, C.A. AND THOMPSON, A.R. (1973). Pesticides and the Soil Fauna. Residue Reviews, **45** (1), 1–73.

EDWARDS, C.A., THOMPSON, A.R. AND LOFTY, J.R. (1968). Changes in soil invertebrate populations caused by some organophosphorus insecticides. Proceeding of 4th British Insecticide and Fungicide Conference, 48–55.

ESCRITT, J.R. (1955). Calcium arsenate for earthworm control. Journal of the Sports Turf Research Institute **9**.

GERARD, B.M. (1960). The biology of certain British earthworms in relation to environmental conditions. PhD Thesis, University of London.

GOATS, G.C. AND EDWARDS, C.A. (1988). The Prediction of Field Toxicity of Chemicals to Earthworms by Laboratory Methods. In: Earthworms in Waste and Environmental Management, Ed. C.A. Edwards and E.F. Neuhauser. SPB Academic Publishing, The Hague, The Netherlands, 283–294.

GOATS, G.C. (1985). Assessment of the Effects of Toxic Chemicals Upon Earthworms. PhD Thesis, University of London, pp 351.

LOFS-HOLMIN, A. AND BOSTRÖM, U. (1988). The Use of Earthworms and Other Soil Animals in Pesticide Testing. In: Earthworms in Waste and Environmental Management. Ed. C.A. Edwards and E.F. Neuhauser, SPB Academic Publishing, The Hague, The Netherlands, 303–313.

POLIVKA, J.B. (1951). Effect of insecticides on earthworm populations. Ohio Journal of Science. **51**, 195–196.

ROBERTS, B.L. AND DOROUGH, W.H. (1984). Relative Toxicities of Chemicals to the Earthworm *Eisenia foetida*. Environmental Toxicology and Chemistry **3**, 66–78.

SATCHELL, J.E. (1955a). The effects of BHC, DDT and parathion on the soil fauna. Soils and Fertilisers. **18**, 279–285.

SATCHELL, J.E. (1955b). An electrical method of sampling earthworm populations. In: Soil Zoology. Ed. D.K. McEkewan, Butterworths, London 356–364.

SAUNDERS, D.G. AND FORGIE, C.D. (1977). Some effects of phorate on earthworm populations. Proceedings 30th New Zealand Weed and Pest Control Conference, 222–226.

THIELEMANN, U. (1986). Elektrischer Regenwurmfans mitt der Oktette-Methode Pedobiologia **29**, 295–302.

THOMPSON, A.R. AND EDWARDS, C.A. (1974). Effects of Pesticides on Non-Target Invertebrates in Freshwater and Soil. In: Pesticides in Soil and Water. Soil Science Society of America Special Publication **8**, 1974. 341.

THOMPSON, A.R. (1971). Effects of nine insecticides on the numbers and biomass of earthworms in pasture. Bulletin of Environmental Contamination and Toxicology **5**, 577–586.

WAY, M.J. AND SCOPES, N.E.A. (1968). Studies on the persistence and effects on soil fauna of some soil-applied systemic insecticides. Annals of Applied Biology **62**, 199–214.

WHEATLEY, G.A. AND HARDMAN, J.A. (1968). Organochlorine residues in earthworms from arable soils. Journal of Science of Food and Agriculture, **19**, 219–225.